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SCIENCE

Melatonin Inhibition of the Neonatal Pituitary Response to Luteinizing Hormone–Releasing Factor

Abstract. *Neonatal rat anterior pituitary glands treated in organ culture with 1 nanomolar luteinizing hormone–releasing factor (LRF) showed a tenfold increase in medium luteinizing hormone (LH) concentrations over control values. Simultaneous treatment of the glands with 1 nanomolar melatonin significantly reduced the stimulatory effect of LRF on release of LH. This finding indicates that melatonin can act directly on the neonatal pituitary to inhibit the LH response to LRF.*

Numerous studies (*1*) have shown that the antigonadotropic effects of the pineal gland may be mediated in part by melatonin. However, the precise mechanism by which melatonin inhibits reproductive function remains to be resolved. We now report that melatonin can act directly on the pituitary gland to suppress the release of luteinizing hormone (LH) induced by luteinizing hormone–releasing factor (LRF).

The effect of melatonin on the LH response to synthetic LRF (*2*) was studied in organ culture with the use of anterior pituitary glands from 5-day-old female Sprague-Dawley rats. The method of culture used in this study (*3*) is identical to that developed in our laboratory for the culture of pineal glands (*4*). With this system, there is no apparent cellular necrosis in 5-day-old pituitaries incubated for 4 days (*3*).

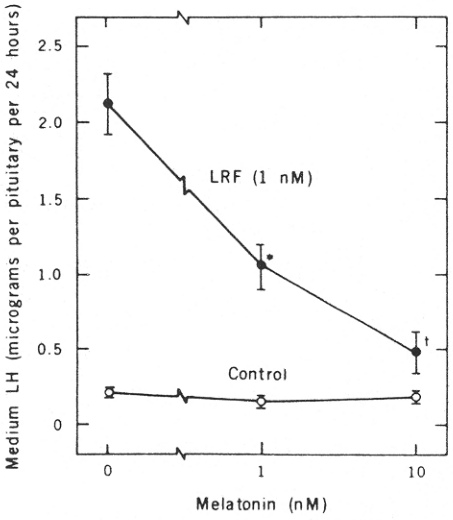

Fig. 1. Effect of melatonin on LRF-induced release of LH. After 24 hours of culture under control conditions, anterior pituitary glands from 5-day-old female rats were treated for 24 hours with control medium, melatonin (1 or 10 n*M*), LRF (1 n*M*), or LRF (1 n*M*) plus melatonin (1 or 10 n*M*). Each point represents the mean response of four anterior pituitaries. Vertical lines represent standard errors. *Significantly less than 1 n*M* LRF and 0 melatonin value; $P < .01$. †Significantly less than 1 n*M* LRF and 0 melatonin value; $P < .001$.

After 24 hours of culture under control conditions, pituitary glands were transferred to fresh medium containing the test substances. Half of the glands were stimulated with LRF (1 n*M*) either alone or in the presence of melatonin (1 or 10 n*M*). The remaining half of the glands served as controls and were incubated either in control medium or in medium containing only melatonin (1 or 10 n*M*). The culture was terminated after a 24-hour treatment period. The LH content of the medium was measured by double antibody radioimmunoassay, in which materials supplied by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases were used. Values are expressed in terms of the reference preparation NIAMDD-Rat-LH-RP-1. Melatonin in culture medium at a concentration of 1 μM does not interfere with the measurement of LH in our assay (*5*). Statistical analysis was made by Student's *t*-test.

As shown in Fig. 1, LRF treatment in the absence of melatonin produced a tenfold increase in the concentrations of LH in the medium over control values. However, simultaneous treatment of the pituitary glands with LRF and 1 n*M* melatonin resulted in a highly significant ($P < .01$) reduction of LH secretion. At 10 n*M*, melatonin suppressed the LRF-induced release of LH to 14 percent of the response obtained with LRF alone. Melatonin had no detectable effect on control concentrations of LH at either of the doses used.

These results indicate that melatonin can act at the pituitary level to suppress

LRF-induced release of LH. Furthermore, this inhibitory effect is evident in vitro at physiologic concentrations. When measured either by bioassay (*6*) or by radioimmunoassay (*7*), concentrations of melatonin in human serum attain values at night of approximately 1 n*M*, a dose which was shown in the present study to inhibit LH release. Other studies in our laboratory (*8*) have demonstrated that the in vitro response to melatonin is rapid, as evidenced by complete suppression of LRF-induced release of LH during a 90-minute incubation period. In addition, specificity of the response in organ culture is indicated by the finding (*8*) that two compounds closely related to melatonin, *N*-acetylserotonin and 5-methoxytryptamine, at concentrations as high at 100 n*M* do not affect the LH response to LRF.

In view of the finding that in the adult rat injection of melatonin into the third ventricle of the brain suppresses LH secretion, whereas injection into a hypophyseal portal vein has no effect on serum LH, Kamberi *et al.* (*9*) have suggested that melatonin inhibits the release of LRF into portal blood. Thus, it appears that melatonin may act both at the hypothalamic level and at the pituitary level to regulate LH secretion. However, their failure to observe suppression of serum LH by melatonin administered directly to the pituitary gland in vivo may be due to the low level of basal LH secretion. It may also be related to the age of the animals. Adult rats were used in their experiments, but neonatal rats were the source of the pituitary glands in our study.

The present finding that LRF stimulation of LH release can be modulated by physiological concentrations of a secretory product of the pineal gland may explain in part the known inhibitory effects of this gland on the reproductive system.

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